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Association between maternal vitamin D status in pregnancy and neurodevelopmental outcomes in childhood; results from the Avon Longitudinal Study of Parents and Children (ALSPAC)

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Abstract

Seafood intake in pregnancy has been positively associated with childhood cognitive outcomes which could potentially relate to the high vitamin-D content of oily fish. However, whether higher maternal vitamin D status [serum 25-hydroxy-vitamin D, 25(OH)D] in pregnancy is associated with a reduced risk of offspring suboptimal neurodevelopmental outcomes is unclear. A total of 7065 mother-child pairs were studied from the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort who had data for both serum total 25(OH)D concentration in pregnancy and at least one measure of offspring neurodevelopment (pre-school development at 6–42 months; "Strengths and Difficulties Questionnaire" scores at 7 years; IQ at 8 years; reading ability at 9 years). After adjustment for confounders, children of vitamin-D deficient mothers (< 50.0 nmol/L) were more likely to have scores in the lowest quartile for gross motor development at 30 months (OR 1.20 95% CI 1.03, 1.40), fine motor development at 30 months (OR 1.23 95% CI 1.05, 1.44), and social development at 42 months (OR 1.20 95% CI 1.01, 1.41) than vitamin-D sufficient mothers (≥ 50.0 nmol/L). No associations were found with neurodevelopmental outcomes, including IQ, measured at older ages. However, our results suggest that deficient maternal vitamin D status in pregnancy may have adverse effects on some measures of motor and social development in children under 4 years. Prevention of vitamin D deficiency may be important for preventing suboptimal development in the first 4 years of life.

Introduction

The consumption of fish, or nutrients present in fish, by pregnant women has been linked to neurocognitive development in their children. In observational studies, maternal intake of fish or seafood in pregnancy has been positively associated with cognitive scores in the offspring[1; 2; 3; 4], while children whose mothers had eaten oily fish in early pregnancy had a reduced risk of hyperactivity than those whose mothers did not eat oily fish[3]. While these studies tended to interpret these associations as effects of of long-chain omega-3 fatty acids, they might also be explained by the fact that oily fish is the best dietary source of vitamin D. Though the action of sunlight on the skin is the predominant contributor to vitamin D status, dietary vitamin D can play an important role in determining status, as measured by the vitamin D metabolite, 25-hydroxyvitamin D [25(OH)D][1], in serum or plasma[5]. Dietary sources of vitamin D (especially oily fish) are particularly important during the winter months when endogenous production of vitamin D status is limited.

It is biologically plausible that vitamin D status in pregnant mothers may affect child neurocognitive development as vitamin D receptors are present in the brain[6] and maternal vitamin D deficiency is known to be associated with abnormal brain development in the young rat[7]. In the period from birth to weaning in rats, there appears to be a window during which maternal vitamin D status affects offspring brain development[8] and these developmental changes may not occur if vitamin D is withheld until weaning[9]. Furthermore, vitamin D deficiency in late gestation can lead to impaired brain function in adult rats[8]. Due to differences between rat and human developmental physiology, the extent to which these findings would apply to humans remains unclear.

Few human studies have assessed the relationship between maternal vitamin D status and neurodevelopmental outcomes. The results of the five published observational studies that exist are inconsistent[10; 11; 12; 13; 14]. Indeed, this fact was recently highlighted in the report from Public Health England on Vitamin D and Health from the Scientific Advisory Committee for Nutrition (SACN) [15].

To address this lack of consistent evidence with respect to the association between maternal vitamin D status and cognitive-developmental outcomes in the offspring, we analysed data from the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort. Our *a priori* hypothesis was that poorer maternal vitamin D status, as measured by serum 25(OH)D, would be associated with

increased probability of suboptimal cognitive or behavioural development scores in childhood of 6 months to 9 years.

Subjects and Methods

Study Design and Participants

Details of ALSPAC methods have been detailed previously [16]. In brief, all pregnant women living in the former Avon area in southwest England, who had an expected delivery date between April 1st 1991 and December 31st 1992 were eligible for inclusion. A total of 14,541 women were recruited, and there were 13,617 mother-child pairs with singleton offspring alive at one year. The ALSPAC study website contains details of all the data that are available through a fully searchable data dictionary (http://www.bris.ac.uk/alspac/). Our study sample consisted of mother-child pairs that had both a serum 25(OH)D measure in pregnancy and at least one neurodevelopmental outcome of interest from 6 months to 9 years (**Figure 1**). A range of outcomes was explored, including motor development, communication and social skills, behaviour, cognition and reading ability.

Outcomes

The ALSPAC pre-school development tests, which were based on questionnaires completed by the mother when the child was between 6 and 42 months of age, provided scores for four domains: fine motor, gross motor, social development, and communication (details published previously[1]). The Strengths and Difficulties Questionnaire (SDQ)[17] was completed by mothers when the child was 81 months of age and was used to assess behavioural development. Intelligence Quotient (IQ) at age 8 years had been assessed in the ALSPAC clinic using the abbreviated form of the Wechsler Intelligence Scale for Children, as previously described [1]. Reading ability (accuracy, comprehension and speed) was assessed at age 9 years by trained psychologists using the Neale Analysis of Reading Ability[18] and by asking children to read real words to derive a reading score. Further details of these outcomes are available in the Supplementary File.

Maternal vitamin D status

Although 25(OH)D has lower biological activity than the active vitamin D hormone, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], serum/plasma 25(OH)D is widely regarded as the most reliable marker of vitamin D status[19]. Total maternal serum 25(OH)D concentration (including both vitamin D2 and vitamin D3) in ALSPAC mothers had been measured in a previous study by high-performance liquid chromatography and tandem mass-spectrometry, in accordance with

Vitamin D External Quality Assessment Scheme (DEQAS) requirements; full details have been published previously[20], including details of inter-assay coefficients of variation[21].

Statistical analysis

The women with vitamin D measurements were compared to the remaining ALSPAC women. We compared categorical variables with χ^2 tests and continuous variables with independent t-tests. We used median (IQR, Inter-quartile Range) to describe maternal vitamin D status. Our main analysis dichotomised women as deficient or sufficient using 25(OH)D concentration $\leq$ 50.0 nmol/L as the cut-off for vitamin D deficiency, as in previous ALSPAC work[20]. We did additional supplementary analyses by dividing women into three categories (< 25.0, 25.0–49.9 and ≥ 50.0 nmol/L) to explore the dose-response relationship.

We used logistic regression to examine the relationship between maternal vitamin D status in pregnancy and odds of suboptimal development with the women in the vitamin-D-sufficient group (> 50.0 nmol/L) as the reference category. We did not input missing confounder or outcome data with replacement values. We defined suboptimal development as scores in the lowest quartile for all subscales of early development, IQ and reading ability, as in previous ALSPAC research [1; 22]. For the SDQ, suboptimal behaviour was defined according to published cut-offs (for both the individual scales and overall score) that indicate borderline/abnormal behaviour [17] (see Supplementary File Study Outcomes). Model predictors were assessed for potential multicollinearity. For our final model, variance inflation factor ranged from 1.02 to 2.2 (accordingly tolerance ranged from 0.5-0.99) depending on the variable.

As vitamin D status and childhood cognitive and behavioural development are affected by a range of factors[23; 24], we included potential confounders in our analysis. The confounders chosen were based on previous ALSPAC findings[1; 22] and were from questionnaire and clinic-based data (**Table 1**). We included ten categorical and two continuous variables. The two continuous variables were maternal age (years), and maternal body mass index (BMI, Kg/m^2). As there is a well-established relationship between BMI and 25(OH)D concentration[25], maternal BMI was included in the model, even though it was not statistically associated with 25(OH)D in this dataset (Table 1).

The ten categorical variables comprised three groups: (i) child factors [gender and breastfeeding (none or some)], (ii) maternal factors [ethnicity (white or non-white), tobacco use in the first trimester (smoker or non-smoker), parity (zero, one or more) and oily fish intake in pregnancy

(never/rarely or once a fortnight or more)], and (iii) markers of socio–economic development [maternal education (low = less than O-level or equivalent; medium = O-level, and high = greater than O-level), home ownership (mortgaged/owned, privately rented or housing association/council rented/other), maternal social class based on her occupation (non-manual and manual) and crowding in the home (≤ one person or > one person per room)]. We also included two variables to control for variation in the vitamin D measurement: gestation (week) and season of sample collection [spring (March, April, and May), summer (June, July, and August) autumn (September, October, and November), and winter (December, January and February)]. While it is unlikely that the age of the child at assessment would be confounded by maternal vitamin D status, outcomes were adjusted for child age at the 6-month measurement, owing to the strong association between age and outcomes at this early life stage.

We used three models to adjust the analysis for potential confounders. As 25(OH)D measurements spanned pregnancy, and as gestational week is associated with vitamin D status[26], we do not present unadjusted data; our minimally adjusted model (Model 1) included gestational week of 25(OH)D measurement. Model 2 built on Model 1 by including nine confounders associated with both vitamin D status (Table 1) and cognitive development (parity, tobacco smoking, housing status, crowding, maternal age, BMI, education, ethnic group, and social class) and two child factors (gender and breastfeeding). Model 3 included Model 2 confounders plus two variables (oily fish intake and season of vitamin D measurement) that could affect maternal vitamin D status though including these may represent an over-control.

We used simulations to assess the impact of multiple comparisons. We generated 5000 datasets where 25(OH)D measurements were randomly permutated across valid observations with these data. As a consequence, all analyses maintained the same number of observations and, with all other data unchanged, the correlations between outcomes and confounders were preserved. The analyses were based upon Model 3. The effect of randomisation was to generate a set of results under the null hypothesis to which our set of observed results could be compared. A composite score across the 27 outcomes was based upon the sum of P values. These were modified to one-sided tests to allow results in the same direction to contribute consistently to the score, whether statistically significant or not. P values in the tables are not corrected for multiple comparisons.

Sensitivity analysis

We conducted analyses with two additional confounders (added to Model 3) that might be on the causal pathway: preterm birth (< 37 weeks or ≥ 37 weeks) and birth weight (< 2500 g or ≥ 2500 g). We also explored the effect of including maternal iodine status in the first trimester [sufficient (≥ 150 μg/g) or deficient (< 150 μg/g)] as we have previously shown that this is associated with child cognition in the ALSPAC cohort[22]. As just 787 women also had a measure of iodine status in the first trimester, we used a simplified model (total of 13 confounders) to ensure that the model would converge (we dropped ethnicity and crowding in the home as a result of low numbers in the categories of those variables).

As there is ongoing controversy in the published literature with respect to the definition of vitamin D deficiency[27], we conducted sensitivity analyses using a wide range of vitamin D status, namely < 25.0 and < 75.0 nmol/L as cut–offs (Supplementary Tables 3 and 4). Assumptions concerning statistical significance were based on interpretation of confidence intervals, rather than P values, wherever possible, and multiple testing was assessed as described above. Analyses were conducted using the Statistical Package for Social Sciences (version 21·0; SPSS, Inc., Chicago, USA).

Ethics

The ALSPAC study was conducted according to the guidelines laid down in the Declaration of Helsinki. All procedures involving human subjects were approved by the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. Written informed consent was obtained from participants (or from their parent/guardian if under 18 years old).

Role of the funding source

The funding bodies did not have a role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the study data used and final responsibility to submit for publication.

Results

Compared with the remainder of the ALSPAC cohort (defined as mother-singleton child pairs from the core sample surviving to one year), the mother-child pairs in this study were more likely to be older, of white ethnicity, with markers of higher socio-economic status [e.g. a higher proportion of breast-feeding mothers, higher educational attainment and social class, and a lower proportion of smokers (Supplementary Table 1)]. However, some of the actual differences were small (e.g.

maternal age 28.3 (4.8) *vs.* 27.7 (4.7) years). The median (IQR) 25(OH)D concentration for all 7065 women with a child that had at least one relevant outcome was 61.3 (42.9 – 84.7) nmol/L, with 4.4% having < 25.0 nmol/L, 34.6% having < 50.0 nmol/L and 65.7% having < 75.0 nmol/L.

The median (IQR) gestational week of vitamin D measurement (available for 7064 women) was 29.6 (12.7, 33.3) weeks, with 26.1% in the first trimester (≤ 13 weeks), 11.8% in the second trimester (14 – 27 weeks) and 62.1% in the third trimester (≥ 28 weeks). The median (IQR) 25(OH)D measurement was 54.9 (40.1 - 72.5) nmol/L in the first trimester, 59.3 (38.6 - 84.2) nmol/L in the second trimester and 65.3 (45.2 - 90.4) nmol/L in the third trimester. Table 1 shows the confounders associated with maternal vitamin D status using the 50 nmol/L cut-off. Women with 25(OH)D concentration ≥ 50.0 nmol/L were more likely to be white, older, and have markers of higher socio-economic status (for example education, home ownership and reduced smoking and crowding).

Results of logistic regression models using the cut-off value for serum 25(OH)D of <50.0 nmol/L to define deficiency are shown in **Table 2**. In the minimally adjusted analysis (Model 1), the only outcomes associated with vitamin D status were verbal IQ at 8 years and words read per minute at age 9 (Table 2). However, after adjustment for potential confounders, the effect on IQ and reading was attenuated and the only outcomes that remained statistically significant were gross- and fine-motor development at 30 months and social development at 42 months. With further adjustment for oily-fish intake and season (Model 3), the association between maternal vitamin D status and gross-motor development also became significant at 18 months, while remaining associated with gross-motor and fine-motor development at 30 months and social development at 42 months (Table 2). Children born to mothers with 25(OH)D ≤ 50**.**0 nmol/L were more likely to have scores in the bottom quartile for these variables.

For the ALSPAC pre-school development assessments, when the serum 25(OH)D of < 50.0 nmol/L group was divided into < 25.0 and 25.0 – 49.9 nmol/L, there was evidence of a statistically significant trend to decreasing risk of suboptimal development with higher maternal 25(OH)D concentration for gross-motor skills at 18 (P=0.02) and 30 months (P=0.008), fine-motor skills at 30 months (P=0.01) and social development at 42 months (P=0.02), after adjustment for all 12 confounders in Model 3 (**Table 3**). The effect sizes were larger for odds of suboptimal development in children of mothers in the serum 25(OH)D < 25.0 nmol/L group, than for the serum 25(OH)D of

25.0 – 49.9 nmol/L group (with the ≥ 50.0 nmol/L group as the comparison group) for all outcomes except fine-motor development at 18 months and social development at 30 months.

The interaction between gestational week of 25(OH)D measurement and the vitamin D variable (i.e. deficient *vs.* sufficient status) was significant for only two of 27 outcomes: fine-motor skills at 30 months and performance IQ (**Table 4)**. However, when the analysis was restricted to the ALSPAC pre-school development assessments and was split into early (≤22 weeks) and late gestation (> 22 weeks), the results suggested that the effect of deficient *vs.* sufficient vitamin D status on the majority of tests was greater in the second half of gestation. The effect sizes were generally larger in the second half of gestation and results were significant (Table 4) for gross motor development at 18 months (Odds Ratio (OR) 0.97, 95% CI 0.76, 1.23 *vs*. OR 1.31, 95% 1.08, 1.58) and 30 months (OR 1.07, 95% CI 0.84,1.38 *vs* OR 1.28, 95% CI 1.05,1.57), fine motor development at 30 months (0.99, 95%CI 0.76,1.29 *vs OR.* 1.37, 95% CI 1.12,1.67) and social development at 42 months (OR 1.07, 95% CI 0.82,1.41 *vs.* OR 1.28, 95% CI 1.03,1.58). There were no significant associations in either half of gestation for other neurodevelopmental outcomes, including the SDQ, IQ or reading ability (Table 4).

Multiple comparisons

While only 4 results in Table 2 were nominally significant at the 5% level, it was noted that 25 of the 27 results in Model 3 showed a detrimental effect for low vitamin D status. Such a result would be highly significant ($p<0.0001$) if the outcomes were independent. In practice, outcomes were correlated with an average $r = 0.12$ (range –0.03 to 0.69). The impact of these correlations was assessed using simulations. The scores from the 5000 simulated datasets had a mean (SD) of 13.52 (2.78). This compared to an expected mean (SD) of 13.5 (1.5) if all the outcomes had been independent. The observed results had a score of 6.93 suggesting an empirical two-tail P value of 0.016. Sequential analyses by removing those outcomes with the strongest association from the simulated scores suggested that three outcomes (gross and fine motor development at 30 months and social development at 42 months) had robust associations with the other 24 outcomes having associations consistent with chance ($p=0.051$).

We also explored defining the score based upon the logit transformation, $\ln(p/(1-p))$. Using this definition, the score more closely approximated to a normal distribution. However this did not change the conclusions.

Sensitivity analysis

When we added the variables, preterm birth and birth weight, to Model 3, the results were fundamentally unchanged (Supplementary Table 2), though the effect of maternal vitamin D status on gross motor development at 18 months and social development at 42 months was no longer statistically significant.

The addition of suboptimal iodine-to-creatinine ratio in the first trimester to Model 3 resulted in considerable sample attrition given the low number of women with iodine measurements (n=787) (Supplementary Table 2). Though the effect sizes were larger than previously, the associations between maternal vitamin D and gross motor development at 18 and 30 months and social development at 42 months were no longer significant, though they remained significant for fine motor development at 18 (OR 1.50, 95% CI 1.02, 2.23) and 30 months (OR 1.61, 95% CI 1.06, 2.46).

We explored whether dichotomising women according to different 25(OH)D cut-offs (25.0 or 75.0 nmol/L) changed the results (Supplementary Tables 3 and 4), bearing in mind the lower relative statistical power that results when the cut-off leads to unequal numbers in each group (the 50.0 nmol/L cut-off was close to the median 25(OH)D concentration of 54.9 nmol/L). When using the 25.0 nmol/L cut-off, the only outcome associated with vitamin D deficiency in the fully adjusted model was gross motor development at 30 months (OR 1.43 95% CI 1.01-2.02); results approached statistical significance for other outcomes (e.g. social development at 42 months, OR 1.40 95% CI 0.97-2.02; Supplementary Table 3). Using a cut-off of 75.0 nmol/L to define deficiency resulted in null associations with the ALSPAC pre-school development assessments, behaviour and cognitive tests, but was associated with higher odds of sub-optimal reading accuracy at 9 years (OR 1.26 95% CI 1.01, 1.57); however, this may be a chance finding as reading accuracy was not associated with vitamin D in any other analyses (Tables 2, 3 and 4 and Supplementary Tables 2 and 3).

Discussion

After adjustment for potential confounders, children born to vitamin-D deficient mothers (serum 25(OH)D of <50.0 nmol/L) were more likely to have sub-optimal gross-motor skills at 30 months, sub-optimal fine-motor skills at 30 months and sub-optimal social development scores at 42 months than were children born to sufficient mothers (≥50.0 nmol/L). Although the effect sizes were relatively small, we consider that the findings were biologically meaningful. Interestingly, no

associations were found between maternal vitamin D status and other outcomes (IQ, reading ability).

These results suggest that the vitamin D content of seafood might explain some of the beneficial effects of maternal seafood consumption seen previously in ALSPAC, at least for fine-motor skills at 30 months and social skills at 42 months[1]. The classification of maternal seafood consumption by Hibbeln et al.[1] included white fish and shellfish which are not good sources of dietary vitamin D, therefore, we would not expect vitamin D intake to account totally for their findings. Furthermore, our results cannot explain previous associations found in ALSPAC between maternal seafood consumption and IQ[1] or between maternal iodine status and IQ and reading ability[22].

Our findings on fine- and gross-motor skills support previous non-ALSPAC-based research that found a positive association between maternal vitamin D status and infant psychomotor development[11]. Although we did not specifically measure scholastic achievement, the lack of an association between maternal vitamin D status and either reading ability or IQ in our study reinforces the findings of a previous study that found no relationship between maternal 25(OH)D status and offspring scholastic achievement[10]. While a US study found a relationship between maternal vitamin D status and offspring IQ, the effect estimates were very small and there was very little indication of an association between maternal blood 25(OH)D and cognitive development, achievement, or behaviour between 8 months and 7 years of age[12].

Our findings suggest that some specific aspects of early neurocognitive development may be suboptimal if maternal prenatal vitamin D is deficient (i.e. serum 25(OH)D of < 50.0 nmol/L) in pregnancy. The biological mechanism underpinning this association in humans is not fully understood, but the ubiquitous presence of the vitamin D receptor (VDR) and the hydroxylase enzymes controlling vitamin D metabolism in a wide variety of areas of the human brain[6], as well as neurological developmental mechanisms previously identified in studies of vitamin D deficiency in pregnant rats may be relevant[7; 9; 28; 29]. These include enlarged brain ventricles, thinner neocortex[29], and more mitotic cells in the brain[29], suggesting a less differentiated phenotype[28]. The active form of vitamin D [1,25$(OH)_2$D], may also affect the development of the brain by influencing the production of cytokines[30], affecting neurotransmission[31] and synaptic plasticity[31] which is likely to affect learning processes[32] and therefore neurocognitive development. 1,25$(OH)_2$D likely affects dopamine activity in the brain owing to the presence of the vitamin D receptor (VDR) in brain areas responsive to dopamine[33]. Ventral midbrain dopaminergic neurones

are known to play a key role in the modulation of motor behaviour[34]. It is therefore feasible that $1,25(OH)_2D$ may affect motor development *via* its effects on the dopaminergic system. Other potential mechanisms may relate to an association between maternal 25(OH)D status and fetal growth retardation (e.g. reduced fetal head size) which is associated with later developmental disabilities[35]. A recent study in the Generation R cohort in the Netherlands found an association between lower maternal 25(OH)D status at 20 weeks gestation and smaller fetal-head circumference in the third trimester[36], suggesting that poorer maternal 25(OH)D status may predispose children to developmental delay *via* effects on intra-uterine growth restriction.

When we assessed the impact of gestational age on our results for outcomes that were significantly associated with vitamin D in the main analyses, we found that the effect sizes were generally greater when vitamin D was measured in the second half (> 22 weeks) than in the first half (≤ 22 weeks) of pregnancy. There is a small amount of evidence in rats that re-introduction of vitamin D after birth, but before end of weaning, can rescue normal brain development[28]; that time period correspond to the third trimester in humans, suggesting a potential crucial window for vitamin D in brain development. However, all interpretations in our analysis of gestational timing need to be interpreted in light of the fact that we only had one measurement of maternal vitamin D status for each woman and so we cannot draw clear conclusions on the effects of gestational timing of vitamin D deficiency. Furthermore, we cannot be sure that our observed effects are confined to the gestational week that the 25(OH)D measurement was made, as some individuals may have persistent pattern of vitamin D status that extends into later pregnancy or infancy.

When the women were split into three groups [serum 25(OH)D of <25.0, 25.0 – 49.9 and ≥ 50.0 nmol/L], adverse outcomes were present in the offspring of mothers with insufficient status (serum 25(OH)D < 50nmol/L) as well as those with severe deficiency (serum 25(OH)D < 25nmol/L). However, there was a trend to larger effect sizes in the more deficient < 25.0 nmol/L group than in the 25.0 – 49.9 nmol/L group; the relatively small sample size in the < 25.0 nmol/L group explains the wider confidence intervals seen for this cut-off. The outcomes that were significantly associated with vitamin D when women were dichotomised on the basis of a cut-off of 50.0 nmol/L were not significant when the cut-off was increased to 75.0 nmol/L. These findings support a vitamin D status cut-off for optimal child outcomes closer to 50.0 nmol/L than to 75.0 nmol/L.

As the women in the ALSPAC study were recruited over 20 years ago, we compared their vitamin D status to more recent measurements in UK women to assess the current relevance of our findings.

As 25(OH)D status does not differ between pregnant and non–pregnant women[15] we looked at nationally representative data in UK women from the recent National Diet and Nutrition Survey (NDNS). In the latest report (sampling 2008/9 – 2011/12), 21.7% of women of 19–64 years had a plasma 25(OH)D concentration below 25 nmol/L[37], a higher percentage than the 4.4% of women in ALSPAC. Other studies[38; 39], including those in pregnancy, suggest that many UK women are vitamin D deficient. Currently, the UK National Institute for Health and Care Excellence (NICE) recommends that pregnant women should take a supplement of 10 µg (400 IU) of vitamin D per day[40]. However use of vitamin D supplements in pregnancy is low, with a recent survey (2005–2009) finding that only 1.4% of UK pregnant women had taken a vitamin D supplement[41]. Our findings give further evidence that public-health campaigns should address the vitamin D status of UK pregnant women, and encourage compliance with the 10 µg/d recommendation[40].

Strengths and Limitations

Although our study has several strengths, including the large sample size, there are also limitations. Firstly each woman had only one measure of maternal vitamin D status in pregnancy which may not have reflected status over the whole of pregnancy. In addition, the range of vitamin D status in the ALSPAC women was limited, with approximately one third (34.6%) having a 25(OH)D concentration less than 50.0 nmol/L and only a small proportion having a 25(OH)D concentration less than 25.0 nmol/L (4.4%). Moreover, ALSPAC only has a relatively small number of women from ethnic-minority backgrounds (just 2% of this study sample), who are known to be at particular risk of having low 25(OH)D concentrations[42], suggesting that the results may differ in populations with a larger number of ethnic-minority individuals. Finally, we were not able to control for the association between infant vitamin D status and neurocognitive function as we had no measures of vitamin D status in infancy. Infant vitamin D status may partly explain some of the association seen in this paper between maternal vitamin D status and infant neurodevelopment.

In conclusion, we found that maternal vitamin D status in pregnancy was associated with a number of adverse neurocognitive developmental variables in early childhood, albeit with a small, but nonetheless important, effect size. There is a need for replication of this work in other settings to confirm these results, but the public-health implications of these findings are nevertheless potentially important. Further study is now urgently required, particularly in population groups that are more severely vitamin D deficient such as dark-skinned ethnic-minority women[37] who may show a wider range and greater severity of sub-optimal neurocognitive outcomes.

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Conflict of Interest

SLN is Research Director of D3Tex Ltd which holds the UK Patent (Gulf Cooperation Council Patent pending) on the use of ultraviolet-B (UVB) transparent material for vitamin D deficiency prevention. All other authors declare that they have no conflicts of interest.

Authors' Contributions to the Manuscript

ALD, SCB and JG designed the current research project. SCB and ALD conducted the statistical analyses with statistical advice from CDS, MPR and JG. MPR, JG, CDS and SLN revised the paper and made suggestions on the content. ALD and SCB wrote the paper. SCB has primary responsibility for final content.

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Table 1 Relationship between confounders and maternal Vitamin D status

Confounder	Maternal vitamin D status						
	< 50.0 nmol/L			≥ 50.0 nmol/L			
	Mean	SD	n	Mean	SD	n	p value†
Age of mother (yrs)	27.7	4.8	2443	28.6	4.7	4622	< 0·0001
BMI of mother (Kg/m^2)	23.0	4.0	2126	22.9	3.6	4095	0.43
Gestation of vitamin D measure (weeks)	23.4	10.9	2771	25.7	10.3	5174	< 0·0001
	%	n		%	n		p value ‡
Breastfeeding							
Some	33·0%	1738		67·0%	3526		< 0·0001
None	38·8%	553		61·2%	874		
Crowding in the home							
< one person per room	33·9%	2140		66·1%	4170		< 0·0001
One or more per room	43.6%	176		56·4%	228		
Education of mother							
Low	37·5%	716		62·5%	1195		< 0·0001
Medium	33·4%	792		66·6%	1577		
High	31·5%	755		68·5%	1643		
Ethnicity of mother							
White	33·3%	2171		66·7%	4344		< 0·0001
Non–white	60·6%	83		39·4%	54		
Gender of child							
Male	34·3%	1266		65·7%	2421		0·67
Female	34·8%	1177		65·2%	2201		
Housing status							
Owned/mortgaged	32·8%	1705		67·2%	3487		< 0·0001
Other rented	36·6%	150		63·4%	260		
Council rented	41·0%	491		59·0%	708		
Iodine–to–creatinine ratio in 1st trimester							
<150 μg/g (deficient)	33.5%	186		66.5%	374		0.94
≥150 μg/g (sufficient)	33.2%	76		66.8%	151		
Oily fish intake in pregnancy (/week)							
Never/rarely	37·7%	1038		62·3%	1718		< 0·0001
Once per fortnight or more	31·3%	1191		68·7%	2617		
Parity							
Zero	37·0%	1125		63·0%	1914		< 0·0001
One or more	31·9%	1179		68·1%	2516		
Season of vitamin D measure							
Spring	48.8%	980		51.2%	1027		< 0·0001
Summer	15.2%	268		84.8%	1491		
Autumn	22.4%	363		77.6%	1257		
Winter	49.5%	831		50.5%	847		
Smoking in 1st trimester							
No tobacco	31·7%	1652		68·3%	3567		< 0·0001
Smoked tobacco	42·5%	689		57·5%	932		
Social class of mother							
Manual	36·6%	383		63·4%	664		0·01
Non–manual	32·5%	1447		67·5%	3008		

† p value from independent t-test.
‡p value for $\chi 2$ test.

Table 2 Odds of suboptimal outcomes according to maternal vitamin D status (< 50.0 vs ≥ 50.0 nmol/L), minimally and fully adjusted for potential confounders

			Model 1†			Model 2‡			Model 3§		
		Age	OR (95% CI)	p value	n	OR (95% CI)	p value	n	OR (95% CI)	p value	n
ALSPAC pre–school development assessments	Gross Motor Skills	6 mo ‖	0.96 (0.84, 1.09)	0.49	6242	1.01 (0.86, 1.18)	0.92	4383	0.96 (0.81, 1.13)	0.59	4380
		18 mo	0.98 (0.87, 1.10)	0.74	6269	1.10 (0.96, 1.27)	0.18	4385	1.17 (1.01, 1.36)	0.04	4383
		30 mo	1.02 (0.91, 1.16)	0.71	5843	1.16 (1.00, 1.34)	0.05	4135	1.20 (1.03, 1.40)	0.02	4133
		42 mo	0.99 (0.87, 1.13)	0.89	5695	1.04 (0.89, 1.22)	0.60	4073	1.09 (0.92, 1.28)	0.31	4070
	Fine Motor Skills	6 mo ‖	0.93 (0.82, 1.05)	0.24	5880	1.07 (0.92, 1.25)	0.39	4141	1.06 (0.91, 1.25)	0.47	4139
		18 mo	1.07 (0.96, 1.21)	0.24	6268	1.03 (0.90, 1.19)	0.65	4383	1.09 (0.94, 1.27)	0.26	4381
		30 mo	1.09 (0.96, 1.23)	0.18	5854	1.20 (1.04, 1.40)	0.02	4138	1.23 (1.05, 1.44)	0.01	4136
		42 mo	1.04 (0.92, 1.19)	0.51	5692	1.11 (0.95, 1.31)	0.19	4071	1.16 (0.98, 1.37)	0.08	4068
	Social Development	6 mo ‖	0.96 (0.84, 1.09)	0.52	6010	1.02 (0.87, 1.19)	0.81	4209	1.00 (0.85, 1.18)	0.98	4207
		18 mo	1.01 (0.89, 1.15)	0.86	6268	1.10 (0.94, 1.28)	0.22	4383	1.14 (0.97, 1.34)	0.11	4381
		30 mo	0.97 (0.86, 1.10)	0.64	5843	1.11 (0.95, 1.30)	0.18	4129	1.07 (0.91, 1.27)	0.42	4127
		42 mo	1.04 (0.92, 1.18)	0.54	5689	1.19 (1.02, 1.39)	0.03	4069	1.20 (1.01, 1.41)	0.04	4066
	Communication	6 mo ‖	0.99 (0.85, 1.15)	0.90	6100	0.99 (0.83, 1.20)	0.95	4285	0.99 (0.81, 1.20)	0.90	4283
		18 mo	0.99 (0.87, 1.12)	0.85	6279	1.11 (0.96, 1.29)	0.17	4390	1.12 (0.95, 1.31)	0.18	4388
Behaviour	Prosocial	7 yr	0.92 (0.75, 1.13)	0.40	4791	0.97 (0.75, 1.24)	0.78	3513	1.00 (0.77, 1.31)	0.98	3511
	Peer problems	7 yr	1.05 (0.88, 1.25)	0.58	4785	1.03 (0.83, 1.27)	0.80	3510	1.05 (0.83, 1.31)	0.70	3508
	Hyperactivity	7 yr	1.06 (0.91, 1.24)	0.47	4780	1.04 (0.86, 1.26)	0.68	3513	1.04 (0.85, 1.26)	0.74	3511
	Emotional	7 yr	1.17 (0.98, 1.41)	0.09	4785	1.14 (0.92, 1.42)	0.23	3511	1.20 (0.95, 1.51)	0.12	3509
	Conduct	7 yr	1.13 (0.99, 1.30)	0.08	4790	1.05 (0.88, 1.24)	0.60	3514	1.06 (0.89, 1.27)	0.50	3512
	Total Score	7 yr	1.08 (089, 1.32)	0.42	4777	1.13 (0.89, 1.44)	0.31	3510	1.24 (0.96, 1.60)	0.09	3508
Cognition	Verbal IQ	8 yr	1.19 (1.02, 1.39)	0.03	3997	1.08 (0.89, 1.31)	0.47	2952	1.00 (0.82, 1.23)	0.98	2950
	Performance IQ	8 yr	1.06 (0.91, 1.24)	0.43	3990	0.99 (0.82, 1.20)	0.92	2945	1.00 (0.82, 1.23)	0.98	2943
	Total IQ	8 yr	1.16 (1.00, 1.35)	0.06	3978	1.02 (0.84, 1.24)	0.82	2938	1.01 (0.82, 1.24)	0.93	2936
Reading ability	Words per min	9 yr	1.17 (1.00, 1.36)	0.05	3794	1.14 (0.94, 1.39)	0.18	2763	1.15 (0.94, 1.42)	0.17	2761
	Accuracy	9 yr	1.16 (0.99, 1.35)	0.07	3802	1.04 (0.85, 1.28)	0.69	2767	1.03 (0.83, 1.27)	0.80	2765
	Comprehension	9 yr	1.11 (0.95, 1.30)	0.18	3802	1.02 (0.83, 1.25)	0.87	2767	1.04 (0.84, 1.29)	0.73	2765
	Reading Score	9 yr	1.10 (0.95, 1.27)	0.22	4125	1.06 (0.88, 1.27)	0.54	3028	1.04 (0.86, 1.26)	0.69	3026

mo, month; OR, odds ratio; n, number of subjects; yr, years. Suboptimal outcome defined as scores in the bottom quartile for ALSPAC pre–school development assessments, cognition, and reading ability. Published cut-offs[17] were used for behaviour: Prosocial (≤5; 9·8%), Peer problems (≥3; 13·5%), hyperactivity (≥6; 18·7%), emotional symptoms (≥4; 12·2%), conduct problems (≥3; 24·3%), and total score (≥14; 10·5%). Maternal vitamin D status >50.0 nmol/L was the reference group. †Model 1 adjusted for gestational week of vitamin D measurement; ‡Model 2: gestational week of vitamin D measurement plus additional 11 variables: maternal age, maternal BMI, maternal ethnic group, maternal education, maternal social class, parity, tobacco smoking in 1st trimester, home ownership status, crowding index, child gender, breastfeeding; §Model 3: additionally adjusted for oily fish and season of vitamin D measurement; ‖ age of child at development test included in all models.

Table 3 Odds of suboptimal outcomes in offspring according to maternal vitamin D status when the < 50.0 nmol/L group is split into < 25.0 and 25.0 – 49.9 nmol/L and each group is compared to ≥ 50.0 nmol/L (adjusted model 3).

			Maternal vitamin D status (nmol/L)					
			< 25.0 *vs.* ≥ 50.0		25.0 – 49.9 *vs.* ≥ 50.0		Trend	
			OR (95% CI)	n	OR (95% CI)	n	p value	n
ALSPAC pre–school development assessments	Gross Motor Skills	6 mo†	1.30 (0.90, 1.88)	169	0.92 (0.77, 1.09)	1279	0.88	4380
		18 mo	1.40 (1.00, 1.96)	178	1.14 (0.98, 1.33)	1270	0.02	4383
		30 mo	1.52 (1.07, 2.17)	163	1.17 (0.99, 1.37)	1213	0.008	4133
		42 mo	1.24 (0.85, 1.82)	159	1.07 (0.90, 1.27)	1191	0.23	4070
	Fine Motor Skills	6 mo†	1.24 (0.85, 1.80)	167	1.04 (0.88, 1.24)	1213	0.32	4139
		18 mo	1.03 (0.72, 1.47)	177	1.10 (0.94, 1.29)	1269	0.36	4381
		30 mo	1.30 (0.91, 1.88)	163	1.22 (1.04, 1.44)	1214	0.01	4136
		42 mo	1.31 (0.89, 1.92)	158	1.14 (0.96, 1.36)	1191	0.06	4068
	Social Development	6 mo†	1.02 (0.70, 1.50)	170	1.00 (0.84, 1.19)	1216	0.95	4207
		18 mo	1.28 (0.88, 1.85)	177	1.12 (0.95, 1.33)	1269	0.08	4381
		30 mo	0.91 (0.61, 1.36)	163	1.09 (0.92, 1.30)	1212	0.66	4127
		42 mo	1.49 (1.02, 2.18)	158	1.16 (0.98, 1.38)	1190	0.02	4066
	Communication	6 mo†	1.41 (0.93, 2.14)	167	0.94 (0.77, 1.16)	1237	0.59	4283
		18 mo	1.31 (0.92, 1.88)	179	1.09 (0.93, 1.29)	1272	0.11	4388
Behaviour	Prosocial	7 yr	1.11 (0.59, 2.09)	124	0.99 (0.75, 1.30)	1003	0.89	3511
	Peer problems	7 yr	0.97 (0.56, 1.67)	124	1.05 (0.84, 1.33)	1002	0.80	3508
	Hyperactivity	7 yr	0.63 (0.37, 1.08)	124	1.09 (0.89, 1.33)	1002	0.70	3511
	Emotional	7 yr	0.80 (0.43, 1.49)	124	1.25 (0.99, 1.57)	1002	0.34	3509
	Conduct	7 yr	0.80 (0.50, 1.27)	124	1.10 (0.91, 1.32)	1003	0.88	3512
	Total Score	7 yr	0.68 (0.33, 1.39)	124	1.31 (1.02, 1.70)	1001	0.37	3508
Cognition	Verbal IQ	8 yr	1.07 (0.67, 1.73)	103	0.99 (0.80, 1.23)	839	0.90	2950
	Performance IQ	8 yr	1.40 (0.89, 2.20)	104	0.96 (0.78, 1.18)	837	0.56	2943
	Total IQ	8 yr	1.37 (0.87, 2.17)	103	0.97 (0.78, 1.20)	834	0.54	2936
Reading ability	Words per min	9 yr	1.11 (0.68, 1.80)	101	1.16 (0.94, 1.43)	797	0.23	2761
	Accuracy	9 yr	1.14 (0.70, 1.87)	101	1.02 (0.81, 1.27)	799	0.69	2765
	Comprehension	9 yr	1.01 (0.61, 1.66)	101	1.04 (0.84, 1.30)	799	0.78	2765
	Reading Score	9 yr	0.91 (0.57, 1.45)	108	1.06 (0.87, 1.29)	872	0.88	3026

mo, month; OR, odds ratio; n, number of subjects; yr, years. Suboptimal outcome defined as scores in the bottom quartile for ALSPAC pre–school development assessments, cognition, and reading ability. Published cut-offs[17] were used for behaviour: Prosocial (≤5; 9·8%), Peer problems (≥3; 13·5%), hyperactivity (≥6; 18·7%), emotional symptoms (≥4; 12·2%), conduct problems (≥3; 24·3%), and total score (≥14; 10·5%). Maternal vitamin D status ≥ 50.0 nmol/L was the reference group. †age of child at development test included in all models.

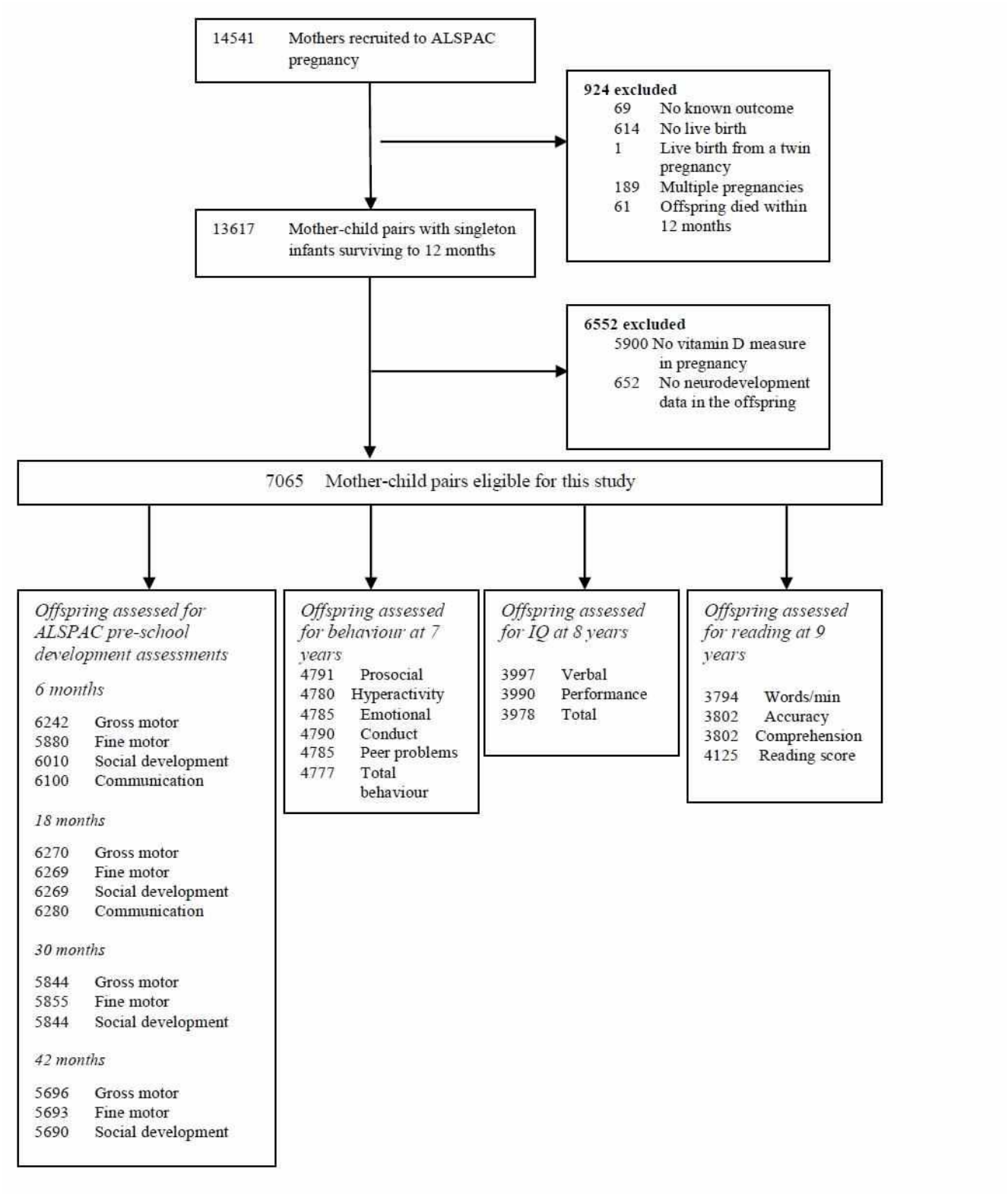
Table 4 Odds of suboptimal outcomes in offspring by maternal vitamin D status (< 50.0 *vs* ≥ 50.0 nmol/L) according to whether maternal vitamin D was measured in the first or second half of gestation (Adjusted Model 3)

			First half of gestation (≤ 22 weeks)			Second half of gestation (> 22 weeks)			P value for interaction*
			OR (95% CI)	P value	n	OR (95% CI)	P value	n	
ALSPAC pre–school development assessments	Gross Motor Skills	6 mo†	0.92 (0. 70, 1.22)	0.56	1500	0.98 (0.79, 1.21)	0.84	2880	0.21
		18 mo	0.97 (0.76, 1.23)	0.78	1522	1.31 (1.08, 1.58)	0.005	2861	0.13
		30 mo	1.07 (0.84, 1.38)	0.58	1435	1.28 (1.05, 1.57)	0.02	2698	0.79
		42 mo	1.03 (0.79, 1.34)	0.85	1422	1.10 (0.89, 1.36)	0.37	2648	0.72
	Fine Motor Skills	6 mo†	1.09 (0.83, 1.44)	0.52	1436	1.03 (0.83, 1.27)	0.80	2703	0.25
		18 mo	1.05 (0.82, 1.36)	0.69	1522	1.10 (0.90, 1.33)	0.35	2859	0.46
		30 mo	0.99 (0.76, 1.29)	0.95	1436	1.37 (1.12, 1.67)	0.002	2700	0.05
		42 mo	1.03 (0.78, 1.37)	0.83	1420	1.24 (1.00, 1.53)	0.05	2648	0.37
	Social Development	6 mo†	0.88 (0.66, 1.16)	0.37	1453	1.11 (0.90, 1.38)	0.32	2754	0.90
		18 mo	1.23 (0.95, 1.60)	0.12	1522	1.07 (0.87, 1.32)	0.51	2859	0.11
		30 mo	0.96 (0.74, 1.26)	0.79	1431	1.13 (0.91, 1.40)	0.28	2696	0.36
		42 mo	1.07 (0.82, 1.41)	0.62	1420	1.28 (1.03, 1.58)	0.02	2646	0.26
	Communication	6 mo†	0.90 (0.65, 1.23)	0.50	1468	1.04 (0.81, 1.34)	0.75	2815	0.37
		18 mo	1.27 (0.98, 1.65)	0.07	1524	1.04 (0.85, 1.28)	0.71	2864	0.17
Behaviour	Prosocial‡	7 yr	0.75 (0.48, 1.17)	0.21	1216	1.15 (0.83, 1.61)	0.40	2301	0.10
	Peer problems	7 yr	1.14 (0.78, 1.66)	0.49	1210	0.97 (0.73, 1.30)	0.86	2298	0.55
	Hyperactivity	7 yr	0.95 (0.68, 1.33)	0.75	1213	1.10 (0.86, 1.41)	0.46	2298	0.31
	Emotional‡	7 yr	1.25 (0.87, 1.80)	0.23	1214	1.17 (0.87, 1.58)	0.29	2301	0.71
	Conduct	7 yr	1.13 (0.84, 1.52)	0.42	1212	1.04 (0.82, 1.31)	0.74	2300	0.76
	Total Score‡	7 yr	1.20 (0.79, 1.82)	0.40	1214	1.24 (0.90, 1.71)	0.18	2300	0.79
Cognition	Verbal IQ	8 yr	1.09 (0.77, 1.55)	0.64	1025	0.93 (0.72, 1.21)	0.60	1925	0.20
	Performance IQ	8 yr	1.15 (0.83, 1.59)	0.42	1017	0.89 (0.68, 1.16)	0.38	1926	0.03
	Total IQ	8 yr	1.18 (0.84, 1.66)	0.33	1015	0.90 (0.69, 1.17)	0.43	1921	0.13
Reading ability	Words per min	9 yr	1.41 (1.00, 1.97)	0.05	936	1.00 (0.77, 1.31)	0.98	1825	0.20
	Accuracy	9 yr	1.31 (0.92, 1.87)	0.13	938	0.87 (0.66, 1.14)	0.32	1827	0.06
	Comprehension	9 yr	1.09 (0.77, 1.55)	0.62	938	0.98 (0.75, 1.29)	0.89	1827	0.31
	Reading Score	9 yr	1.30 (0.94, 1.78)	0.11	1060	0.91 (0.71, 1.16)	0.44	1966	0.20

mo, month; OR, odds ratio; n, number of subjects; yr, years. Suboptimal outcome defined as scores in the bottom quartile for ALSPAC pre–school development assessments, cognition, and reading ability. Published cut-offs[17] were used for behaviour: Prosocial (≤5; 9·8%), Peer problems (≥3; 13·5%), hyperactivity (≥6; 18·7%), emotional symptoms (≥4; 12·2%), conduct problems (≥3; 24·3%), and total score (≥14; 10·5%). Maternal vitamin D status ≥ 50.0 nmol/L was the reference group and Model 3 was used (without gestational week of vitamin D assessment as this was used to split analyses). *interaction between vitamin D (deficient/sufficient) and gestational week of sample (continuous variable); †age of child at development test included in all models; ‡ethnicity removed as model would not converge.

Legends for Figures

Figure 1: Flow of participants


14541 Mothers recruited to ALSPAC pregnancy
924 excluded
69 No known outcome
614 No live birth
1 Live birth from a twin pregnancy
189 Multiple pregnancies
61 Offspring died within 12 months
13617 Mother-child pairs with singleton infants surviving to 12 months
6552 excluded
5900 No vitamin D measure in pregnancy
652 No neurodevelopment data in the offspring
7065 Mother-child pairs eligible for this study
Offspring assessed for ALSPAC pre-school development assessments
6 months
6242 Gross motor
5880 Fine motor
6010 Social development
6100 Communication
18 months
6270 Gross motor
6269 Fine motor
6269 Social development
6280 Communication
30 months
5844 Gross motor
5855 Fine motor
5844 Social development
42 months
5696 Gross motor
5693 Fine motor
5690 Social development
Offspring assessed for behaviour at 7 years
4791 Prosocial
4780 Hyperactivity
4785 Emotional
4790 Conduct
4785 Peer problems
4777 Total behaviour
Offspring assessed for IQ at 8 years
3997 Verbal
3990 Performance
3978 Total
Offspring assessed for reading at 9 years
3794 Words/min
3802 Accuracy
3802 Comprehension
4125 Reading score

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